Published in final edited form as:

Acta Biomater. 2010 August ; 6(8): 3349–3359. doi:10.1016/j.actbio.2010.01.046.

Porous Tantalum Structures for Bone Implants: Fabrication, Mechanical and *In vitro* Biological Properties

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Abstract

Relatively high cost of manufacturing and inability to produce modular all tantalum implants has limited its widespread acceptance, in spite of its excellent *in vitro* and *in vivo* biocompatibility. In this article, we report how to process Ta to create net shape porous structures with varying porosity using Laser Engineered Net Shaping (LENS™) for the first time. Porous Ta samples with relative densities between 45 to 73% have been successfully fabricated and characterized for their mechanical properties. *In vitro* cell materials interactions, using human osteoblast cell line hFOB, have been accessed on these porous Ta structures and compared with porous Ti control samples. The results show that the Young's modulus of porous Ta can be tailored between 1.5 to 20 GPa by changing the pore volume fraction between 27 and 55%. *In vitro* biocompatibility in terms of MTT assay and immunochemistry study showed excellent cellular adherence, growth and differentiation with abundant extracellular matrix formation on porous Ta structures compared to porous Ti control. These results indicate that porous Ta structures can promote enhanced/early biological fixation. The enhanced *in vitro* cell-materials interactions on porous Ta surface are attributed to chemistry and its high wettability and surface energy relative to porous Ti. Our results show that these laser processed porous Ta structures can find numerous applications, particularly among older patients, for metallic implants because of their excellent bioactivity.

Keywords

Porous metals; Tantalum; Laser processing; *In vitro* biocompatibility; Bone implants

1. Introduction

Metallic biomaterials currently in use for load bearing orthopedic applications are bioinert and lack in sufficient osseointegration required for implant longevity [1–3]. One consideration to improve the healing process is focused on the use of bioactive calcium phosphate ceramics on metal implants. Unfortunately, cracking, delamination and low fracture toughness of ceramic coatings still remain some of the concerns for its widespread application in load bearing metal implants. In the last two decades, a variety of porous coatings and materials have also been used to obtain biological fixation of bone with metal implants in load bearing implants. The most common metallic materials include Ti and CoCrMo alloy sintered beads, diffusion

bonded Ti, fiber metal, and plasma spray Ti [1–4]. Clinical and histological evidence from retrieved implants clearly demonstrate that these porous surfaces enhance bone tissue ingrowth and are effective in supplementing the stability of the implant by biological fixation [5–7]. Table 1 reviews some of the characteristics of common porous materials pertinent to orthopaedics that are commercially available. These implants can be reliably and economically manufactured and implanted in a facile and reproducible manner. However, it is also important to note that majority of these materials are porous coatings applied on fully dense substrates. These porous materials are best suited for use as coatings since they do not readily have the required mechanical properties that would allow them to be used as bulk structural materials for implants, bone augmentation, or substitutes for bone graft. Moreover, these porous coated Ti alloy implants show 50 to 75% lower fatigue strength compared to their equivalent fully dense materials [8–10], which arises due to highly stress concentrated regions at particle-substrate neck regions acting as crack initiation sites. Other perceived limitations of these porous metals and processing routes include: the desire for controlled porosity characteristics, relatively high modulus of coatings, difficult to make stand-alone structures and limited part geometries and sizes. Some of these limitations have been addressed by developing open cell metal structures. The overall manufacturing process involves creating a reticulated skeleton with deposition of a metal onto the surface. Trabecular Metal™ is one such open cell porous Ta marketed by Zimmer Inc. (Warsaw, IN, USA). It is being made from the pyrolysis of a thermosetting polymer foam creating a low-density vitreous carbon skeleton. Then commercially pure Ta is deposited onto this interconnected scaffold using chemical vapor deposition/infiltration [11,12]. One major limitation of these open cell porous scaffolds is lack of sufficient mechanical strength to be used as load bearing metal implants. Therefore, porous metals fabrication technologies, which can ensure tailorable pore size, shape and distribution, net shape fabrication capability, sufficient mechanical strength and high levels of purity for biomedical applications assumes significant importance.

Among metallic biomaterials, tantalum is gaining more attention as a new biomaterial. Tantalum has been shown to be corrosion resistant [13] and bioactive *in vivo* [14]. Through the formation of a bone-like apatite layer in simulated body fluid, tantalum has been shown to be bioactive and biologically bond to bone. In several *in vitro* and animal studies, the porous Ta metal has provided a scaffold for bone ingrowth and mechanical attachment [11,15–17]. These porous Ta components offer a low modulus of elasticity, high surface frictional characteristics, and excellent osseointegration properties (i.e., bioactivity, biocompatibility, and in-growth properties) [18–20]. However, extremely high melting temperature of Ta (3017°C), besides its high affinity towards oxygen, makes it difficult, if not impossible, to process Ta structures via conventional processing routes. Although Trabecular Metal™ is commercially available, relatively high cost of manufacture and inability to produce a modular all tantalum implant has limited its widespread acceptance. The development of porous tantalum is in its early stages of evolution and in this work, we have fabricated net shape, bulk porous Ta structures using high power lasers in Laser Engineered Net Shaping (LENS™). Specific advantages of using laser processing in conjunction with LENS™ are (i) ability to control melting of high melting point Ta thus creating fully dense or tailored porosity implants, (ii) net-shape fabrication of complex shaped implants, (iii) retention of high purity of initial feed stock power in the final product, (iv) fine and uniform microstructural features as a result of localized melting and subsequent solidification improving mechanical, physical and biological properties. In the recent years, we have extensively used LENS™ to create fully dense, porous, compositionally and structurally graded materials for load bearing metal implants [21–29]. Moreover, our recent *in vitro* biocompatibility study, using human osteoblast cell line hFOB, on laser deposited Ta coatings on Ti showed six times higher living cell density, excellent cellular adherence and growth with abundant extracellular matrix formation on Ta coating surface compare to Ti surface [30]. This is an important finding as such a high number of living cell density during *in vitro* biocompatibility evaluation has not been reported on any

of the currently used metallic biomaterials. Therefore, to fully utilize the excellent biocompatibility of Ta, for load bearing metal implants, suitable processing techniques have to be developed. In this context, the current article is reporting the LENSTM processing of porous Ta structures and their mechanical and *in vitro* biological properties.

2. Materials and Methods

2.1 Fabrication and Characterization of Porous Ta

Ta metal powder (Grandview Materials Inc., Columbus, OH) with 99.5% purity and particles size between 45 and 75 μm was used. Porous Ta samples with 7.5 mm diameter and 10.5 mm height were deposited on a substrate of 3 mm thick rolled, commercially pure (CP) Ti plates using LENSTM-750 (Optomec Inc. Albuquerque, NM) equipped with a 500W continuous wave Nd:YAG laser. Detailed description of LENSTM operation and capabilities can be found elsewhere [21,23,24,27]. Porous Ta samples were fabricated in a glove box containing an argon atmosphere with O₂ content less than 10 ppm to limit oxidation of Ta during processing. Laser powers of 125 and 350 W were chosen to partially melt metal powders during the deposition process to create the porous structures. Scan speeds between 10 and 20 mm/s were used to fabricate structures with varying porosity. Similarly, powder feed rates of 126 and 141 g/min were used to vary the porosity in the samples. Also, the distance between two successive metal roads or laser scans was varied between 0.381 and 1.27 mm to tailor the pore size. Porous Ta samples with three different densities have been produced using different process parameters, which are shown in Table 2. The total energy input per unit volume (E) as a function of processing parameters was evaluated from [31]:

$$E = \frac{P}{v \cdot h \cdot t} \quad (1)$$

where, P is laser power, v is scan speed, h is hatch distance or scan line spacing and t is Z-increment or layer thickness.

At each process parameter combination, three samples were prepared separately for bulk density, open pore volume and mechanical properties evaluation. Statistical analysis was performed on the experimental data using Student's t-test, and $p < 0.05$ was considered statistically significant. Bulk density of the samples was determined by measuring the physical dimensions and the mass of the samples. Apparent density was measured in water using Archimedes principle. The fraction of open and closed pores in the samples was calculated from the bulk and apparent densities. Cross sectional microstructures of the samples were examined using light microscope and Field Emission Scanning Electron Microscope (FE-SEM) (FEI – Quanta 200F). To reveal microstructural features, polished Ta samples were further chemically polished for 30 sec using a solution containing 25 ml lactic acid, 15 ml HNO₃ and 5 ml HF. These chemically polished samples were then etched with 20 ml HNO₃, 20 ml HF and 60 ml H₂SO₄ solution for 60 sec.

Compression tests for mechanical property evaluation were carried out using a servo hydraulic MTS (axial/torsion materials test system) machine with 250 kN capacity at a strain rate of 10^{-3}s^{-1} . Young's modulus and 0.2% proof strength were determined from the stress-strain plots derived from load-displacement data recorded during compression testing. Vickers microhardness measurements were made on the coatings using a 100 g load for 15s, and an average value of 10 measurements is reported.

2.2 In vitro Cell Culture

In vitro cytotoxicity behavior of laser processed porous Ta with 27% and 45% porosity, and porous Ti control sample with 27% porosity was evaluated and compared for a maximum incubation period of 11 days using human fetal osteoblast cell (hFOB) line (CRL-11372, ATCC, VA, USA). All samples were sterilized by autoclaving at 121°C for 20 minutes prior to the cell culture experiment. Following this, cells were seeded onto sample surfaces and placed in a 24-well plate. Initial cell density was 2.0×10^4 cells/wall. 1 ml of DMEM media enriched with 10% fetal bovine serum was added to each well. Based on ATCC's hFOB cell culture protocol, cultures were maintained at 34°C under an atmosphere of 5% CO₂ and 95% air for up to 7 days for cells attachment and proliferation. After 7 days, cells were cultured at 39.5°C under an atmosphere of 5% CO₂ and 95% air for cells differentiation. The culture media were changed every alternate day.

2.2.1 Cell Morphology—Cell morphology was assessed after 3, 7 and 11 days of incubation period under SEM. Cultured samples for SEM observation were rinsed with 0.1 M phosphate-buffered saline (PBS) and fixed with 2% paraformaldehyde/2% glutaraldehyde in 0.1 M cacodylate buffer overnight at 4°C. Following this, post fixation for each sample was performed with 2% Osmium tetroxide (OsO₄) for 2 h at room temperature. Fixed samples were then dehydrated in an ethanol series 30%, 50%, 70%, 95% and 100% three times, followed by a hexamethyldisilane (HMDS) drying procedure. Dried samples were then mounted on aluminum stubs, gold coated and observed under an FESEM.

2.2.2 Cell Proliferation using MTT Assay—Proliferation of viable hFOB cells attached on porous Ta and Ti sample surfaces was assessed by the MTT assay (Sigma, MO, USA) after 3, 7 and 11 days of incubation period. An MTT solution [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium] of 5 mg/ml was prepared by dissolving MTT in PBS, and filter sterilized. MTT solution was diluted (100 µl into 900 µl) with DMEM culture media enriched with 10% fetal bovine and added to each sample to form formazan by mitochondrial dehydrogenases. After 2h incubation at 34°C, samples were transferred to a new 24-well plate and 1 ml of solubilization solution made up of 10% Triton X-100, 0.1 N HCl and isopropanol were added to dissolve the formazan crystals. Then 100 µl of the resulting supernatant was transferred into a 96-well plate and three data points were obtained from each sample. The optical density of the solution in each well was measured at a wavelength of 570 nm using a microplate reader (Cambridge Tech., MA, USA).

2.2.3 Immunochemistry and Confocal Microscopy—Vinculin and alkaline phosphatase (ALP), the specific protein expressions respectively relevant to focal adhesion formation and osteoblast cell differentiation, have been assayed for test samples by fluorescent staining and confocal laser scanning microscopy (CLSM) observation. Briefly, different test samples were cultured for 3 days in the case of vinculin expression study, and 11 days for the alkaline phosphatase (ALP) expression study. After pre-specified days of incubation, cultured samples were rinsed with 0.1 M phosphate-buffered saline (PBS) and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer and kept for 24h at 4°C for future use. Following these, samples were incubated in 0.5% Triton X-100 in 0.1 M phosphate-buffered saline (PBS) for 10 minutes and blocked with TBST/BSA (tris-buffered saline with 1% bovine serum albumin, 250 mM NaCl, pH 8.3) for 1h at room temperature. Primary antibody against alkaline phosphatase (ALP) or vinculin (Sigma, MO, USA) was added at a 1:100 dilution and incubated at room temperature for 2h. Then the samples were rinsed three times with TBST/BSA (tris-buffered saline with 1% bovine serum albumin, 250 mM NaCl, pH 8.3) for 10 minutes. Following this, the secondary antibody, goat antimouse (GAM) oregon green (Molecular Probes, OR, USA), was added at a 1:100 dilution and incubated for 1h. Samples were then mounted on coverslips with Vectashield mounting medium (Vector Labs, Burlingame, CA)

with propidium iodide (PI) and kept at 4°C for future CLSM imaging. Confocal microscopy was performed on a Zeiss 510 laser scanning microscope (LSM 510 META, Carl Zeiss MicroImaging, Inc., NY, USA). In the present study, specific absorption of vinculin or alkaline phosphatase (ALP) is identified by the expression of green fluorescence and nuclei counterstained with a chemical dye propidium iodide (PI) present in the mounting medium expressed as red fluorescence. For green fluorescence excitation Ar ion (488nm, 30 mW) laser was used at 50% of maximum output at full power and for red fluorescence excitation He-Ne (543 nm, 1.2 mW) laser was used at full output and full power. A combination of a band-pass filter transmitting 505–570 nm and a long pass filter at 560 nm was applied to obtain fluorescence images. Since the test samples were opaque, the confocal pinholes were opened up to 560 μm to pass more fluorescence light to improve fluorescence image quality.

2.2.4 Statistical Analysis—MTT assay results are presented as mean $\pm$ standard deviation. Statistical analysis was performed on MTT assay results using student's t-test and a P value < 0.05 was considered significant.

3. Results

3.1 LENSTM Processed Porous Ta Structures

Figure 1a shows the influence of energy input on relative density of LENSTM processed porous Ta samples. The relative density of porous Ta samples varied between 45 and 73% depending on specific energy input which has been changed by tailoring the LENSTM processing parameters. Increasing the energy input from 23.9 J/mm³ to 361.7 J/mm³ increased the density of porous Ta samples from $45.7 \pm 1.7\%$ to $73.2 \pm 2.2\%$. In general, total porosity increased with increasing powder feed rate or scanning speed and decreasing the laser power. Highly porous samples were obtained at a laser power of 125 W, scan speed of 10.8 mm/s, 141 g/min powder feed rate and 1.27 mm hatch distance. Since the open porosity is important for tissue in growth, the amount of open pore volume in the LENSTM processed porous Ta samples were evaluated and Figure 1b shows the variation of total, closed and open porosity observed in porous Ta. It can be seen that in all the samples the amount of open pores was higher than the amount of closed pores. Also, both closed and open pore volume decreased with increasing laser energy input. The open pore volume in present samples varied between 38% and 64% of the total volume % porosity.

Representative cross-sectional, i.e., perpendicular to build direction, microstructures of porous Ta samples showing variations in pore size, distribution and their connectivity are shown in Figure 2a and b. Irrespective of laser process parameters, all samples showed large irregular pores and fine spherical pores. However, the sample with 73% density showed relatively more number of spherical pores than other samples with lower density. The irregular pore diameter in these samples varied between 200 and 2000 μm and fine spherical pores were less than 100 μm in diameter. As expected, largest pore mean diameter was obtained in the samples made with highest hatch distance of 1.27 mm. Pore connectivity increased with decrease in the relative density and increase in the open pore volume fraction. Samples with 45 and 55% relative density showed maximum pore interconnectivity. Figure 2c shows the grain structure of LENSTM processed Ta. The grain size measured using a linear intercept method was between 8 and 15 μm for the samples with 45 and 55% relative density. A significantly larger grain size of 50–70 μm was observed in the samples with 73% density. This indicates that specific energy input has strong influence on both the amount of porosity and grain size in the laser processed Ta samples. However, the average hardness of porous Ta samples with different porosity was within the standard deviation of average hardness of 395 ± 30 Hv.

The 0.2% proof strength and Young's modulus of porous Ta samples as a function of relative density are shown in Figure 3a and 3b, respectively. Mechanical properties increased with

increasing density of the samples. Experimental data show that the modulus of laser processed Ta samples can be tailored in the range of 2 and 20 GPa by controlling the LENSTM process parameters. The 0.2% proof strength of these porous samples was in the range of 100 to 746 MPa.

3.2 In vitro Behavior of Porous Ta Structures

3.2.1 Morphology of hFOB Cells—SEM observation revealed hFOB cells attachment, growth and spreading on different porous Ta and Ti sample surfaces. Figure 4 shows morphologies of cells on samples after 3 days of culture. Cells were flattened and well-spread on porous Ta surfaces after 3 days as shown in Figures 4a–c. On both the 27% porous Ta (Ta-27) and 45% porous Ta (Ta-45) sample surfaces, cells were seen to adhere to each other with cellular micro extensions (e.g. filopodia) and connected to the substrate in addition to the neighboring cells. Moreover, on Ta-45 sample, as shown in a high magnification SEM image Figure 4c, cells were observed to grow into the pores. In contrast, on 27% porous Ti (Ti-27) surface, cell growth was less pronounced, where limited establishment of cell spreading can be noticed after 3 days of culture. From Figure 4d, it can be seen that cells assumed a more round shape with diffused or under developed filopodia on porous Ti surface compared to porous Ta surface. Figure 5 shows cell morphology after 7 days of culture period on porous Ta and Ti surfaces. On Ta-27 surface cell numbers increased and entire sample's surface was covered with cells having numerous filopodia extensions grabbing the surface irregularities as shown in Figure 5a. Figure 5b shows cell morphology on Ta-45, where cells grew in monolayer and formed a flat and well-spread morphology on smooth surface. Also, cells were found to migrate into the pores and observed to bridge the pores. However, on the Ti-27 control surface, relatively fewer cells were observed. SEM images of hFOB cells cultured for 11 days are presented in Figure 6, where a significant difference in cell growth behavior among the samples can be noticed. After 11 days of culture, cells appeared to be more elongated and confluent on Ta-27 as shown in Figure 6a. Evidently, granules were deposited on cells surfaces because of extracellular matrix (ECM) mineralization. In contrast, on Ta-45, cells never reached complete confluence all over the sample surface because of the higher pore volume. As shown in Figure 6b, on Ta-45 samples cell formed multilayer clusters on smooth surface area but could not bridge the large pore areas. Interestingly, ingrowth of mineralized bone cells was observed inside the pores as shown Figure 6c. However, on the Ti-27 control sample surface cells did not form any confluent layer even after 11 days of culture, however, the rough cellular surface and presence of apatite granules, as shown in Figure 6d, indicates some ECM mineralization.

3.2.2. Cell Proliferation—MTT assay was used to quantitatively determine the proliferation of viable hFOB cells on porous Ta and Ti surfaces. Figure 7 shows a comparison of viable cell densities for different samples after 3, 7 and 11 days of culture. Evidently for all culture durations, cells were proliferated in higher numbers on porous Ta samples compared to that observed on porous Ti samples. Although the differences in cell densities were low during initial culture duration, cells grew almost 50% higher in number on Ta samples compared to Ti surface over 7 days of culture period. Statistical analysis revealed that significant difference (P value < 0.05) in cell density was always present between porous Ta, both 27 % and 45% porosity, and porous Ti (27% porous) samples for all culture days. It was observed that during the initial culture days, cells proliferated most rapidly on samples with highest porosity, i.e., on Ta-45, in comparison to Ta-27 samples. However, after 11 days of culture there was no statistically significant difference present in cell densities between Ta-45 and Ta-27 samples.

3.2.3 Immunochemistry and Confocal Microscopy—Osteoblast adhesion and response to material surfaces after 3 days of culture were evaluated by immunochemical analyses using confocal imaging and the results are shown in Figure 8. All samples showed a positive immunostaining for vinculin, a protein expressed by osteoblast cells which forms at

focal adhesion sites. However, the intensity and distribution of vinculin expression were different for different samples depending on the material's chemistry and porosity level. Figure 8a shows a moderately strong green fluorescence for the Ta-27 samples indicating formation of considerable number of focal adhesion sites, which allow cytoskeleton to establish spread shape morphology. In porous Ta samples, increasing the porosity level increased the vinculin protein expression as indicated by a very strong green fluorescence on the Ta-45 surface compared to Ta-27 sample (Figure 8b). In contrast, on Ti-27 sample's surface, fewer cells as well as small amount of vinculin distribution was found as shown in Figure 8c. Relatively weak green fluorescence expression for Ti samples indicates less production of vinculin protein, which resulted in poor cellular attachment and limited spreading.

ALP protein expression was assessed as a differentiation marker for hFOB cells and observed under a confocal microscope. Figure 9 shows the confocal micrographs of ALP expression in hFOB cells after 11 days of cultured period. Ta samples with different porosity level, i.e., 27% and 45%, exhibited very strong green fluorescence signifying positive immunostaining for ALP as shown in Figures 9a and 9b, respectively. In contrast, a moderate ALP expression was observed on 27% porous Ti surface (Figure 9c).

4. Discussion

4.1 LENSTM Processing of Porous Ta Structures

LENSTM processed parts are produced via layer wise deposition, and each layer consists of a number of consecutive overlapping tracks/scans. The final density of a LENSTM processed part can be considered as an average of the density of each track/scan. Therefore, the extent of powder melting in each track/scan determines the achievable porosity in the final part, as a function of laser parameters. Present results show that total porosity of Ta samples can be tailored by changing laser energy input via LENSTM processing parameters. High amounts of porosity observed at low specific energy input is due to lower working temperatures, which partially melts the Ta powders during LENSTM deposition. These partially melted powders join together in the presence of the liquid metal at the particle-particle interfaces, leaving some inter-particle porosity. Particle bonding during LENSTM processing is a direct result of localized melting and subsequent solidification, in contrast to solid state sintering in powder metallurgical route. Therefore, the inherent brittleness associated with solid state sintered metal powders is completely eliminated in laser processing samples. While, increasing the energy input increases the working temperatures and consequently melts the powder completely leading to dense deposits. Melting of powders also depends on feedstock powder size. In general, it is difficult to control melting of fine powder, which can completely melt during deposition leading to denser parts. However, coarse powders, when used, do not completely melt during deposition and can therefore, attain high amount of porosity at identical laser energy input. In our earlier work [21] on LENSTM processing of porous NiTi alloy samples, a specific energy input of $\sim 100 \text{ J/mm}^3$ resulted in only 10% porosity, as compared with 45% in the present Ta samples. Therefore, the amount of melting at specified laser energy input strongly depends on material's laser absorption capacity, its melting temperature and powder particle size. Apart from the total porosity, the open pore volume in porous Ta samples increased with decreasing energy input, which is understandable. Total open pore volume between 38% and 65% of total porosity in the present porous Ta samples allow more body fluids to be transported through the interconnected pores if implanted, which can subsequently accelerate the healing process by allowing tissue to grow inside the implants and improve biological fixation.

Laser processing is characterized by extremely high cooling rates, of the order of 10^3 to 10^5 K/s, which influence several aspects of liquid metal solidification. It is well known that the scale of dendritic/grain structure is inversely proportional to the solidification cooling rates.

Also, the cooling rates depend on energy input and the cooling rates decreases with increasing energy input. Thus, the higher cooling rates during fabrication of 45 and 55% dense Ta samples with low energy input resulted in finer grain than the samples made at high energy input. The high laser energy input used to make 73% dense Ta samples results in lower cooling rates as a result of shallow thermal gradients during deposition leading to coarse grain size. The elastic modulus varied depending on porosity of the structures. The decrease in mechanical properties with an increase in the porosity is intuitive. In general, the modulus of as-processed porous Ta between 2 and 20 GPa closely match that of human cortical bone which has a modulus in the range of 3 to 20 GPa. Modulus matching has the potential to reduce stress-shielding and related problems in load bearing metallic implants.

4.2 In vitro Behavior

Present *in vitro* bone cell-materials interactions results clearly indicate that the porous Ta samples have superior biocompatibility over porous Ti samples with identical porosity, as assessed qualitatively by SEM cell morphology, immunochemistry and confocal microscopy analyses, and quantitatively using MTT assay. Superior cell adherence with numerous cellular micro extensions on porous Ta samples compared to Ti samples clearly suggests that Ta surfaces are biocompatible and cause no inhibition to bone cells (hFOB) adhesion and growth. Presence of relatively high ECM mineralization on porous Ta samples also indicates that osteoblast cells have started differentiating and ECM remodeling [32]. Our results on increased surface occupancy by osteoblast cells on porous Ta are in good agreement with previous studies demonstrating enhanced osteoblast adhesion and proliferation on Ta coated Ti surfaces over pure Ti surface [30]. Observed differences in cell morphology on different surfaces, after 11 days of culture, can be explained on the basis of average pore size of different samples. For the samples with 27% porosity cells can easily form a confluent multilayer covering the entire sample surface due to relatively smaller pore size in these samples than in the samples with high volume fraction of porosity. On Ta samples with 45% porosity, cells can not span directly across the pores as the mean pore size was $> 500 \mu\text{m}$ resulting in coverage of surface with cells. However, in these samples cells can grow into the pores. Similar results on influence of pore size on cell morphologies have been reported [28].

The pore volume fraction and the surface morphology of porous Ta samples have been shown to have significant influence on hFOB cell adhesion, growth, motility, and differentiation. MTT results clearly show that cell attachment and proliferation depends on sample porosity in addition to material chemistry. MTT results show that after 3 days culture period, cells proliferate most rapidly on samples with highest porosity, i.e., Ta-45 (45% porosity) in comparison to 27% porous Ta samples (Ta-27), while 27% porous Ti (Ti-27) exhibits lowest cell density. It is believed that higher surface area and rough surface topography of the pores favors better cellular attachment, which enhances cell proliferation and coverage inside pore areas. Although MTT assay results show no statistically significant difference ($P \text{ value} > 0.05$) in cell densities between Ta-45 and Ta-27 samples after 11 days, highly porous structures are beneficial for bone in growth, and extensive body fluid transport for osseointegration of the implant [33,34].

Immunochemistry and confocal microscopy results show strong green fluorescence indicating strong focal adhesion sites on Ta-45 (45% porosity) samples compared to the other two samples. It is hypothesized that increased surface area of 45% porous Ta samples helped in better anchorage and stronger bonding of the cells to Ta-45 sample surfaces. Moreover, the higher wettability and higher surface energy of Ta (55 mN m^{-1}) than Ti (42 mN m^{-1}) [30] enhanced *in vitro* cell-materials interactions on Ta surfaces as compared with Ti. In general, higher surface energies and lower contact angle supports cell adhesion and spreading to a greater extent than materials that have low surface energies [35]. Relatively weak green

fluorescence expression for Ti samples indicates less production of vinculin protein, which results in poor cellular attachment and limited spreading.

Our *in vitro* results show that porous Ta samples not only enhance cell adhesion and proliferation, but also stimulate cell differentiation in comparison to porous Ti samples. This has been confirmed by enhanced ALP activity on Ta surface, which indicates later stages of differentiated osteoblast function and can be linked to osteoid production and matrix mineralization process [36]. In contrast, a moderate ALP expression on 27% porous Ti surface signifies early stages of hFOB cells differentiation period. High level of ALP expression on porous Ta samples clearly demonstrates that on Ta surface differentiating osteoblasts are in bone remodeling stage after the post-proliferative period of extracellular-matrix maturation and ECM mineralization [37]. These results are in good agreement with previous investigations indicating that Ta causes no denaturation of proteins and thus can permit *in vivo* osseointegration [38].

Our experimental results on fabrication and *in vitro* biocompatibility of porous Ta indicate that modular all tantalum implants with tailored porosity and superior biocompatibility can be fabricated using LENS™. As a result of high wettability and surface energy of Ta early integration of bone tissue with Ta implants can occur.

5. Conclusions

Fabrication of porous Ta structures with total porosities between 27 and 55% has been successfully demonstrated using LENS™. The experimental results show that this process has the potential to create modular full Ta implants with tailored porosity. The Young's modulus and 0.2% proof strength of Ta has been tailored in the range of 2–20 GPa and 100–746 MPa, respectively. The mechanical properties of laser processed porous Ta are similar to those properties of human cortical bone. MTT assay showed significantly higher number of living cell density on Ta samples than Ti samples. Strong cellular attachment and spreading in terms of high intensity and distribution of vinculin expression was observed on laser processed Ta samples. Also, enhanced ALP activity on porous Ta surfaces indicated later stages of cell differentiation in contrast to early stages of hFOB cells differentiation period on porous Ti surfaces. These *in vitro* biocompatibility results show that laser processed porous Ta have significantly better hFOB cell adhesion, growth, motility, and differentiation than porous Ti indicating its enhanced/early bioactivity.

Acknowledgments

The authors would like to acknowledge the Office of Naval Research (Grant No. N00014-1-05-0583), the National Science Foundation (Grant No. CMMI 0728348) and the National Institutes of Health (Grant No. NIH-R01-EB-007351) for the financial support. Financial support from the W. M. Keck Foundation to establish a Biomedical Materials Research Lab at WSU is also acknowledged.

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Figure 1.
Influence of specific energy on (a) relative density and (b) total, open and closed porosity of LENS™ processed Ta samples.

Figure 2. FESEM microstructures of porous Ta structures (a), (b) porosity characteristics in 55 % and 73% dense samples, respectively, (c) grain size of LENSTM processed porous Ta.

Figure 3. Mechanical properties of porous Ta structures as a function of sample's relative density (a) 0.2% proof strength, (b) Young's modulus.

Figure 4. SEM micrographs illustrating hFOB cell morphology after 3 days of culture period on (a) 27 % porous Ta (Ta-27), (b, c) 45 % porous Ta (Ta-45), and (d) 27 % porous Ti (Ti-27) sample surfaces.

Figure 5. SEM micrographs illustrating hFOB cell morphology after 7 days of culture period on (a) Ta-27, (b) Ta-45, and (d) Ti-27 sample surfaces.

Figure 6. SEM micrographs illustrating hFOB cell morphology after 11 days of culture period on (a) Ta-27, (b, c) Ta-45, and (d) Ti-27 sample surfaces.

Figure 7.

MTT assay of cells on 27% porous Ta (Ta-45), 45% porous Ta (Ta-45) and 27% porous control Ti samples after 11 days of incubation time. There were significant differences in optical density (* = $P < 0.05$, $n = 5$) between porous Ti and Ta samples for all time period. However, after 11 days culture period the difference was not significant between Ta-27 and Ta-45 samples (** = $P > 0.05$, $n = 5$).

Figure 8.

Confocal micrographs of vinculin protein expression in hFOB cells on (a) 27 % porous Ta (Ta-27), (b, c) 45 % porous Ta (Ta-45), and (d) 27 % porous Ti (Ti-27) after 3 days of culture period. Green fluorescence indicating antibody bound to vinculin and cell nuclei were contrast-labeled in red by adding propidium iodide (PI).

Figure 9. Confocal micrographs of ALP protein expression in hFOB cells on (a) 27 % porous Ta (Ta-27), (b, c) 45 % porous Ta (Ta-45), and (d) 27 % porous Ti (Ti-27) after 11 days of culture period. Green fluorescence indicating antibody bound to ALP and cell nuclei were contrast-labeled in red by adding propidium iodide (PI).

Table 1

Characteristics of commercial porous materials pertinent to orthopaedics.

Name	Description	Processing	Applications	Limitations
Actipore™	Fully porous NiTi alloy. Porosity ~ 65% Pore Size ~ 215 µm	Not reported	Spine and shoulder surgeries.	Low mechanical properties and limited part geometries.
Regenerex™	Fully porous Ti. Porosity ~ 67% Pore Size ~ 300 µm Modulus ~ 1.6 GPa	Porous plasma processing	Hip and shoulder surgeries.	Low strength, limited sizes and only acetabular components and augments are available
Tritanium™	Porous Ti coating. Porosity ~ 60% Pore Size ~ 616 µm Modulus ~ 106–115 GPa	Low temperature arc vapor deposition of polyurethane foam shell	Hip surgeries	It is not manufactured as a stand-alone structure, and it is only available in acetabular components. The manufacture process is also limited at this time to large sized components
CSTi™	Porous Ti coating. Porosity ~ 50–60% Pore size ~ 400–600 µm, Modulus: 106–115 GPa.	Powder sintering with pressure.	Hip and knee surgeries.	Simple and limited part geometries, low fatigue resistance.
Stiktite™	Porous Ti coating. Porosity ~ 60% Pore Size ~ 500 µm Modulus ~ 100–110 GPa	Porous plasma processing	Hip and knee surgeries.	Simple and limited part geometries, low fatigue resistance.
Trabecular Metal™	Open cell fully porous Ta. Porosity ~ 75–85% Pore Size ~ 550 µm Modulus ~ 2.5–3.9 GPa	Chemical vapor deposition/infiltration of carbon skeleton.	Hip and knee surgeries.	Relatively high cost of manufacture, inability to produce a modular all tantalum implant
CoCr Beads	Porous CoCrMo coating. Porosity ~ 30–50% Pore Size ~ 100–400 µm Modulus ~ 210 GPa	Powder sintering	Hip surgeries.	Simple and limited part geometries, low fatigue resistance.
Fiber Metal	Porous Ti coating. Porosity ~ 40–50% Pore Size ~ 100–400 µm Modulus ~ 106–115 GPa	Sintering	Hip surgeries.	Simple and limited part geometries, low fatigue resistance.

Table 2

LENSTM parameters and mechanical properties of porous Ta samples.

Power, W	Scan Speed, mm/s	Powder Feed Rate, g/min	Layer Thickness, mm	Hatch Distance, mm	Specific Energy, J/mm ³	Density, %	0.2% Proof Strength, MPa	Young's Modulus, GPa
350	10	126	0.254	0.381	361.7	73.2 ± 2.2	746 ± 27	20 ± 1.9
125	20	141	0.127	0.508	96.9	55.3 ± 1.5	192 ± 7	7 ± 0.6
125	10.8	141	0.381	1.27	23.9	45.7 ± 1.7	100 ± 10	1.5 ± 0.3